Phasor Fluorescence Lifetime Microscopy of Free and Protein-Bound NADH Reveals Neural Stem Cell Differentiation Potential.

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Public Summary:
In the stem cell field there is a lack of non invasive and fast methods to identify stem cell's metabolic state and their ability to turn into more specialized cells, such as particular types of brain cells. Here we describe a label-free method that uses a molecule inside the cell, NADH, as an intrinsic biomarker and a specific type of microscopy (the Phasor approach to Fluorescence Lifetime Microscopy, or FLIM) to measure the metabolic fingerprint of cells. We show that different metabolic states are related to whether the stem cells will turn into neuronal or glial brain cells without the expression of other types of markers. Our data demonstrate that the NADH FLIM signature distinguishes a particular brain cell type, neurons, from neural progenitor and stem cells (NPSCs) at two different developmental stages. NPSCs follow a trajectory highlighting two different stages in the metabolic pathway that converts food into energy in the cell as they become more specialized cells. NSPCs are characterized by high free/bound NADH ratios, while neurons are characterized by low free/bound NADH ratios. We demonstrate that the metabolic signature of NPSCs correlates with their potential to form different types of specialized cells, showing that neuronal progenitors and glial progenitors have a different free/bound NADH ratio. Reducing conditions in NPSCs correlates with their ability to form neurons, while oxidative conditions correlate with the formation of glia. For the first time we show that FLIM NADH metabolic fingerprints provide a novel and quantitative measure of stem cell status and a label-free and non-invasive means to identify neuron- or glial- biased progenitors.

Scientific Abstract:
In the stem cell field there is a lack of non invasive and fast methods to identify stem cell's metabolic state, differentiation state and cell-lineage commitment. Here we describe a label-free method that uses NADH as an intrinsic biomarker and the Phasor approach to Fluorescence Lifetime microscopy to measure the metabolic fingerprint of cells. We show that different metabolic states are related to different cell differentiation stages and to stem cell bias to neuronal and glial fate, prior the expression of lineage markers. Our data demonstrate that the NADH FLIM signature distinguishes non-invasively neurons from undifferentiated neural progenitor and stem cells (NPSCs) at two different developmental stages (E12 and E16). NPSCs follow a metabolic trajectory from a glycolytic phenotype to an oxidative phosphorylation phenotype through different stages of differentiation. NSPCs are characterized by high free/bound NADH ratio, while differentiated neurons are characterized by low free/bound NADH ratio. We demonstrate that the metabolic signature of NPSCs correlates with their differentiation potential, showing that neuronal progenitors and glial progenitors have a different free/bound NADH ratio. Reducing conditions in NPSCs correlates with their neurogenic potential, while oxidative conditions correlate with glial potential. For the first time we show that FLIM NADH metabolic fingerprint provides a novel, and quantitative measure of stem cell potential and a label-free and non-invasive means to identify neuron- or glial- biased progenitors.